Study of production of γ-Poly glutamic acid by Bacillus licheniformis

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ABSTRACT

Abstract Medium E(L-glutamic acid 20g/L,citric acid 12g/L,glycerol 80g/L, NH4Cl 7g/L, MgSO4 7H2O 0.5g/L, FeCl3 6H2O 0.04 g/L, K2HPO4 0.5 g/L, CaCl2.2H2O 0.15 g/L, and MnSO4 H2O 0.04 g/L) is a medium of choice for many researchers investigating production of poly- γ -glutamic acid(γ -PGA)by Bacillus species. When Bacillus licheniformis CCRC12826 was cultivated in medium E, the amount of γ -PGA produced was only 5.27g/L, which is much less than that produced by other Bacillus species in the medium. When glutamic acid、citric acid、glycerol and ammonium chloride were substituted by other carbon and nitrogen sources, it was found that the production of γ -PGA by B.lciheniformis CCRC12826 decreased significantly. It was concluded that glutamic acid、citric acid、glycerol and ammonium chloride were still the most suitable carbon and nitrogen sources for the production of γ -PGA by Bacillus licheniformis CCRC12826, Response surface methodology(RSM) was employed to study the effect of glutamic acid, citric acid, glycerol and initial medium pH on the production of γ -PGA by Bacillus licheniformis CCRC12826 shaken cultures. The result of first order RSM showed that the liner terms of glutamic acid, citric acid and glycerol had significant positive effect, but the initial medium pH exhibited no significant effect on γ -PGA. The effects decreased in the order of glycerol, glutamic acid and citric acid. In addition, the interaction term of glutamic acid-glycerol exhibited a significant positive effect. Based on the results of the first-order RSM, the path of steepest ascent was determined to bring the factors level to more close to their optimum level. The results of the path of steepest ascent showed that the highest production response was 14.35 g/L when the concentration of citric acid, glutamic acid and glycerol was 17.9g/L, 55.4g/L, 148.4 g/L respectively. After having climbed up to response surface to regions of higher production response, the optimum composition was then investigated by using a central composite design (CCD). The experimental results of CCD were fitted with a second-order polynomial equation by a multiple regression analysis. The goodness of fit of the model was checked by several criteria. The determination coefficient R2=0.9078 indicated that 9% of the total variance is not explained by the model。 The Fisher F-test is significant at upper 5% level,and the lack of fit is insignificant at 5% level。 All of these indicate a good adequacy of the second-order polynomial model。 The results of this study have show that there is significant 300%, from 5.27g/L to 21g/L, improvement of γ -PGA production by Bacillus licheniformis CCRC12826 through RSM. The maximal γ -PGA yield(21g/L) appeared at the regions where respective concentrations of glutamic acid、citric acid and glycerol were around 65 g/L、22 g/L、170 g/L respectively。

Keywords : γ -Poly(glutamic acid) ; Response surface Methodology ; Bacillus licheniformis

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